Research Article

Bioactive and functional compounds of mixed beverages based on fruits and vegetables

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Abstract
In recent years, the demand for fruits and vegetables has increased in several countries. Thereby, the consumption of natural beverages increased, and specifically those with claims of functional properties. However, there is a clear need for further studies on the beneficial effects of these beverages, and greater attention should be given to brands that use this information in a manner that is unconcerned with consumer health. This work aimed to study the physical–chemical composition and bioactive and functional compounds of some brands of natural drinks marketed in Brazil. Six brands of natural drinks were acquired and analyzed for pH, titratable acidity (%), soluble solids, total sugars, color, total phenolics, vitamin C, β-carotene/linoleic acid, DPPH and dietary fiber. The drinks analyzed are in accordance with the literature for acidity, except the 'E' brand. The sugar content found in three brands ('A,' 'B-Le' and 'D') was higher than reported on the labels, and the 'A' brand misleads the consumer by stating that their drink is 'zero sugar.' In general, the drinks showed yellowish color, except the 'E' brand with greenish coloration. The brand 'A' also had higher levels of vitamin C than established by law. The amount of phenolic compounds was higher in the 'A' and 'F' brands. The antioxidant content was higher in 'A,' 'C' and 'F.' The 'D' and 'F' brands were shown as sources of dietary fiber. The content of some brands analyzed is in disagreement with their labels which represents a danger to consumers with some kind of food restriction.

Keywords Juice · Antioxidants · Dietary fiber · Detox · DPPH

JEL Classification I11

Mathematics Subject Classification 92-02

1 Introduction

Demand for fruit and vegetables has increased in recent years in several countries in response to increased public awareness of the benefits of healthy eating. It has been suggested that consumption of antioxidant-rich foods may delay or prevent many diseases [1, 2]. Therefore, the guide to health promotion and disease prevention in the USA and around the world includes recommendations for daily consumption of a variety of fruits and vegetables, since they have significant amounts of bioactive compounds, especially vitamins, minerals and fiber [3]. To help ingesting adequate amounts of fruits and vegetables, juices are a practical alternative [4].

In order to increase the consumption of natural drinks, fruit juice companies are expanding their niche markets

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into a category that now extends beyond traditional fruit juices like orange and apple for a line of innovative vegetable blends, given the clear trend toward higher consumption of natural and less processed products, and consumers are willing to pay more for products that offer what they are looking for, especially when it comes to health and wellness [5].

There is evidence suggesting that the juices of different fruits contain bioactive components and, having this information, some companies producing natural juices use them on their labels, even claiming detox properties. However, there is a clear need for larger, well-controlled, long-term studies with well-defined results. Most studies had a limited sample size of gender and ethnicity, as well as short treatment periods and focus on early biomarkers rather than expensive, but more meaningful functional results [3, 4].

This paper aims to study the physicochemical composition and bioactive compounds of some brands of natural drinks marketed in Brazil, some of which claim on their labels to have detoxifying properties, in order to know their compositions confronting them with their labels and verify the content of its bioactive compounds.

2 Materials and methods

The eight natural drinks from six different brands were purchased in the Lavras, Minas Gerais, Brazil trade. The brands 'A,' 'C,' 'E' and 'F' were selected for this study because they declare on their label that they are detox, the brand 'B' for being sold as a natural drink and has the term 'DTOX' on its label, inducing consumers to think that it is a detox drink, the brand 'D' was selected because its own name and marketing suggest selling healthiness to its consumers. The brands were coded as 'A,' 'B,' 'C,' 'D,' 'E' and 'F.' The 'B' brand was found in the orange (B-Or), passion fruit (B-PF) and lemon (B-Le) flavors. The description of the presentation, method of preparation, whether or not it is detox, sugar content and dietary fiber given on the package labeling are given in Table 1. Table 2 shows the ingredients of each brand. Among the evaluated brands, five are marketed nationally and only the brand 'E' is marketed in the state of Minas Gerais, Brazil. Only brand 'A' states the amount of vitamin C (45 mg/200 mL) and brand 'B' does not explicitly state that juices are detox, but uses the word 'DTOX' on their packaging. All drinks used were within the expiration date indicated on the packaging/or label. The drinks were taken to the Food Chemical and Biochemical Analysis Laboratory of the Food Science Department of the Federal University of Lavras and evaluated for pH, titratable acidity (%) expressed as citric acid, soluble solids, total sugars, color, total phenolics, vitamin C, β-carotene/linoleic acid, DPPH and dietary fiber.

The pH was determined using a Tec 3MP pH meter (TECNAL) according to the Association of Official Analytical Chemists [6] (AOAC) technique. The titratable acidity was determined by titration with 0.01 mol/L sodium hydroxide (NaOH) solution, using phenolphthalein as an indicator, according to the Adolfo Lutz Institute [7]. Results were expressed as percent citric acid. For the determination of soluble solids, a PAL-1 portable digital refractometer (ATAGO) was used. Results were expressed as percent soluble solids [6]. Total sugars were determined by the antrona method [8]. Results were expressed as a percentage. The coloring was determined on a Minolta CR-400 colorimeter, with determination in CIE mode $L^*a^*b^*$. The L^* coordinate represents how light or dark the sample is, with values ranging from 0 (all black) to 100 (all white), commonly used to check the darkening. The hue angle ($h°$) refers to the tint, commonly used as a synonym for color, where 0° represents pure red, 90° pure yellow, 180° pure green and 270° pure blue. Chroma (C*) is related to the purity of the hue, which varies from intense or highly chromatic (values close to 60) to neutral (white, gray, black), values close to zero [9]. Total phenolics were determined by the Folin–Ciocalteau method [10]. The phenolic content was calculated from the equation of the straight line obtained from the

Table 1 Description of natural drinks according to their labels

	Presentation	Preparation	Detox?	Sugars	Dietary fiber
'A' Brand	Powder	20 g/200 mL	YES	5,0 g/200 mL	No sign.[c]
'B-Or' Brand	Liquid	RC[a]	DTOX[d]	10.0 g/200 mL	No sign.
'B-PF' Brand	Liquid	RC	DTOX[d]	6.0 g/200 mL	No sign.
'B-Le' Brand	Liquid	RC brand	DTOX[d]	6.0 g/200 mL	No sign.
'C' Brand	Power	1.4 g/200 mL	YES	N.D.[b]	No sign.
'D' Brand	Liquid	RC	NO	15.24 g/200 mL	0.33 g/200 mL
'E' Brand	Power	1 g/200 mL	YES	N.D.	N.D.
'F' Brand	Liquid	RC	YES	24 g/200 mL	5.1 g/250 mL

[a]*RC* Ready for consumption, [b]*ND* No declaration, [c]Not significant quantity, [d]This brand does not claim that the product is Detox, but has this written on the front of the label

Table 2 List of ingredients provided on drinks labels. *Source*: Natural drinks label

Ingredients	
'A' Brand	Maltodextrin, dehydrated cabbage powder, dehydrated spinach powder, orange pulp, blueberry pulp, apple pulp, dehydrated parsley powder, citric acid acidulant, guar gum thickener, sucralose sweetener, chlorophyll natural dye, selenium, ascorbic acid, niacinamide (3ibofla), thiamine mononitrate (vitamin B1), riboflavin (vitamin B2), pyridoxine (vitamin B6), cobalamin (vitamin B12), vitamin A acetate (vitamin A), ferric pyrophosphate (iron), folic acid, zinc sulfate (zinc) and flavorings
'B-Or' Brand	Water, whole orange juice, cabbage, mint, spinach, ginger, whole lime juice and citric acid acidulant
'B-PF' Brand	Water, whole orange juice, whole passion fruit juice, apple juice, cabbage, mint, spinach, ginger, whole lime juice and citric acid acidulant
'B-Le' Brand	Water, wholemeal lemon juice, apple juice, cabbage, mint, spinach, ginger, holy grass and citric acid acidulant
'C' Brand	Organic green tea leaf powder (matcha–Camelia sinensis), citric acid acidulant, pineapple-like aroma with mint, maltodextrin, sodium citrate acidity regulator, sucralose and acesulfame-k sweeteners, dehydrated pineapple pulp (pineapple juice, maltodextrin and citric acid acidulant), tricalcium phosphate antiumectant
'D' Brand	Water, pineapple juice and mint
'E' Brand	This brand markets the prepared powder in bulk without informing the ingredients. The instruction of preparation is given by the sellers
'F' Brand	Kiwi, pineapple, concentrated juice (apple, lemon), green tea extract, mint, chlorophyll, fruit pectin stabilizer

standard gallic acid curve. Results were expressed in milligrams of gallic acid equivalent (EAG) 100 mL^{-1} drink. Ascorbic acid content (after oxidation to dehydroascorbic acid) was determined by the colorimetric method using 2,4-dinitrophenylhydrazine according to Strohecker and Henning [11]. The determination of antioxidant activity by the β-carotene/linoleic acid method was performed according to the methodology described by Almeida et al. [12]. Results were expressed as percent inhibition of oxidation. The determination of antioxidant activity by the DPPH radical sequestration method was performed according to the description of Brand-Williams et al. [13], adapted by Rufino et al. [14]. Results were expressed as percentage of free radical sequestration (% SRL). Total dietary fiber (FAT), soluble dietary fiber (FS) and insoluble dietary fiber (FI) were determined by the enzymatic–gravimetric method suggested by AOAC [6]. Results were expressed as percentage of fiber. Results were expressed as mg of ascorbic acid 100 mL^{-1} drink.

Analyzes were performed with three replicates in triplicate. The results were evaluated by univariate statistical analysis (ANOVA) and means test (Scott Knott, $p \leq 0.05$) were performed using the Sisvar software [15].

3 Results

The 'E' brand had a pH higher than 6.0 and this was the highest value found, followed by the 'D,''B-Or' and 'A' brand. The 'B-PF' and 'F' brands had lower pH than the aforementioned brands and were statistically equal. The 'B-Le' and 'C' brands had the lowest pH values (Fig. 1).

To titratable acidity, the 'F' brand had the highest value, followed by the 'B-Le' brand. The drinks 'A,''B-Or' and 'B-PF'

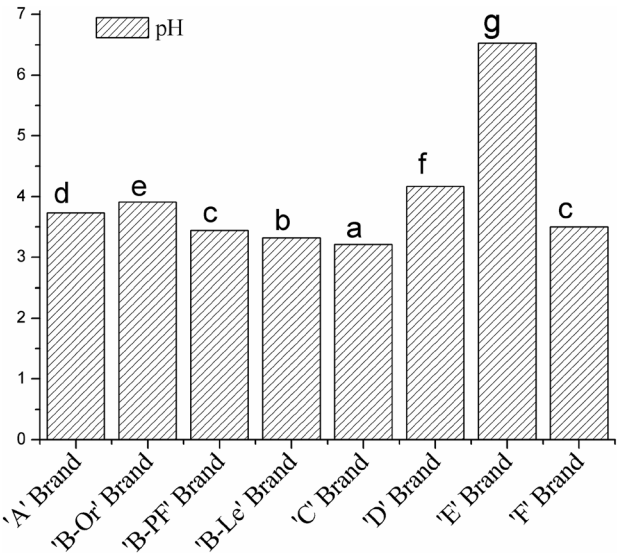

Fig. 1 Mean of pH from six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott-Knott test at 5% significance level

were statistically the same. The brand C and D had lower values than those previously mentioned and were equal to each other. The E brand obtained the lowest titratable acidity value when compared to the others (Fig. 2).

Regarding the soluble solids content, the 'F' brand stood out, followed by the 'D' brand and then by the 'A' and 'B-Le' brands that showed no difference between them. The 'B-Or' brand had the fourth highest average, the 'B-PF' the fifth and the 'C' and 'E' the lowest averages (Fig. 3). As with soluble solids analysis, the 'F' brand had the highest total sugars value followed by the 'D' brand. The 'B-Or' and 'B-Le' brand were equal with third highest average, and the

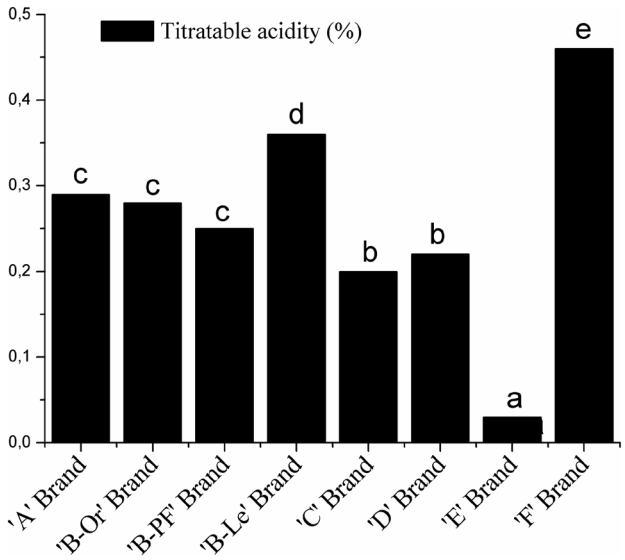

Fig. 2 Mean of titratable acidity (%) values expressed as citric acid from six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott-Knott test at 5% significance level

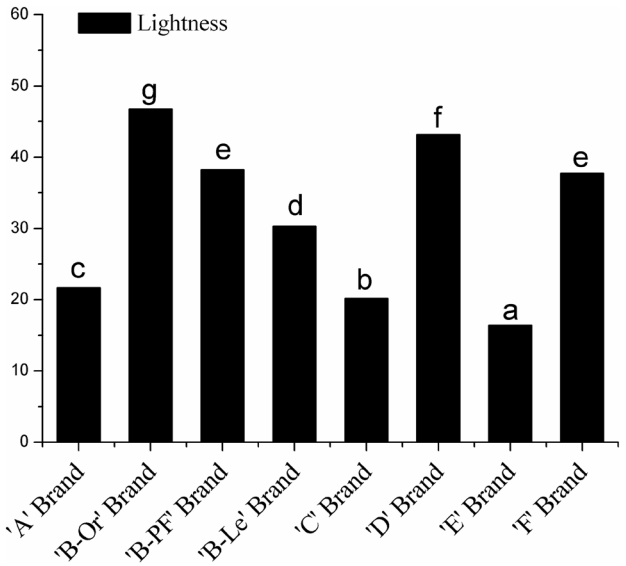

Fig. 4 Average values of lightness of six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott-Knott test at 5% significance level

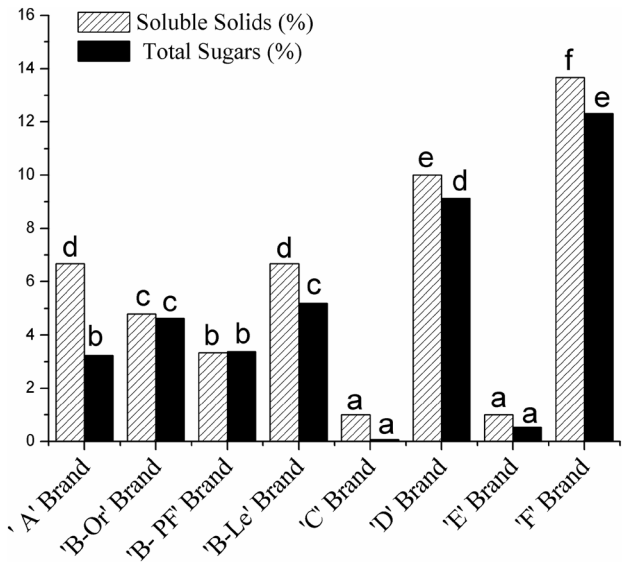

Fig. 3 Average values of soluble solids (%) and total sugars (%) of six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott-Knott test at 5% significance level

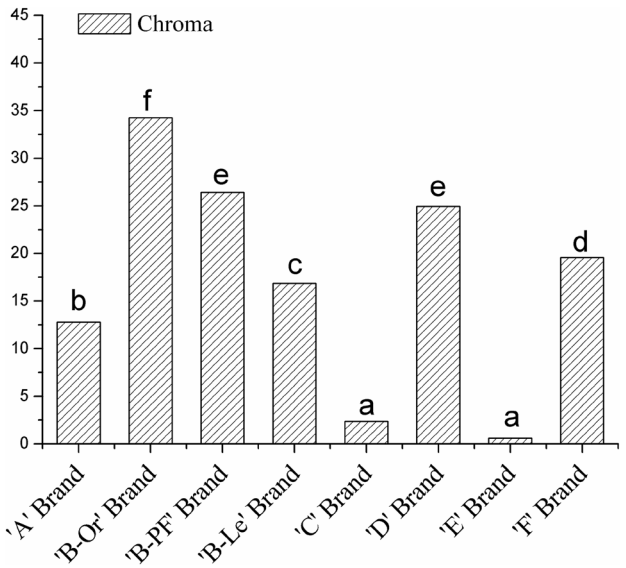

Fig. 5 Average values of chroma of six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott-Knott test at 5% significance level

'A' and 'B-PF' brands showed the fourth highest average and the lowest values were found in the 'C' and 'E' brands (Fig. 3).

The lightest drink was 'B-Or' followed by the 'D' brand (Fig. 4). The brands 'B-PF' and 'F' were equal to each other. The 'A' brand got a higher brightness than 'C' and the 'E' brand was the darkest.

The chroma or color saturation was also higher in the 'B-Or' brand and the 'C' and 'E' brands had the lowest values. The 'B-PF' and 'D' drinks were the same and the other three brands were different from each other (Fig. 5).

Regarding the hue angle, the brand 'E' had the highest average, green color, followed by 'C,' yellowish green color. The other drinks did not vary with yellow color (Fig. 6).

The brand 'A' stood out with respect to the value of total phenolic compounds, which was 2.2 times higher than the second higher brand (F). The 'D' brand scored the third highest average, and this was higher than the 'C' and 'B-Or'

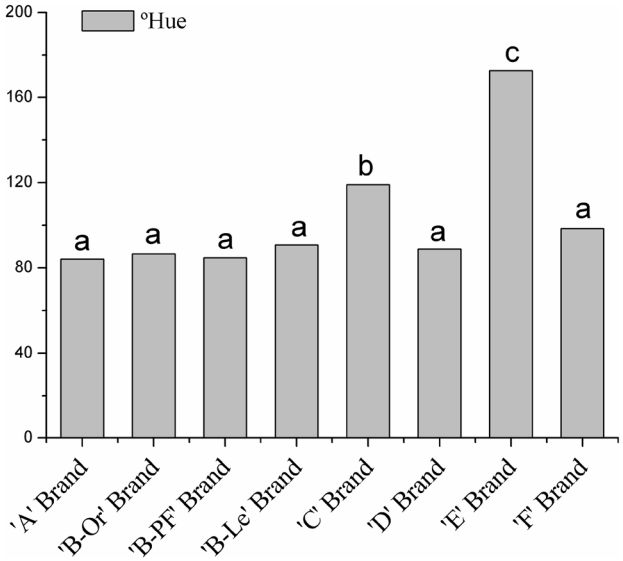

Fig. 6 Average values of °hue of six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott- Knott test at 5% significance level

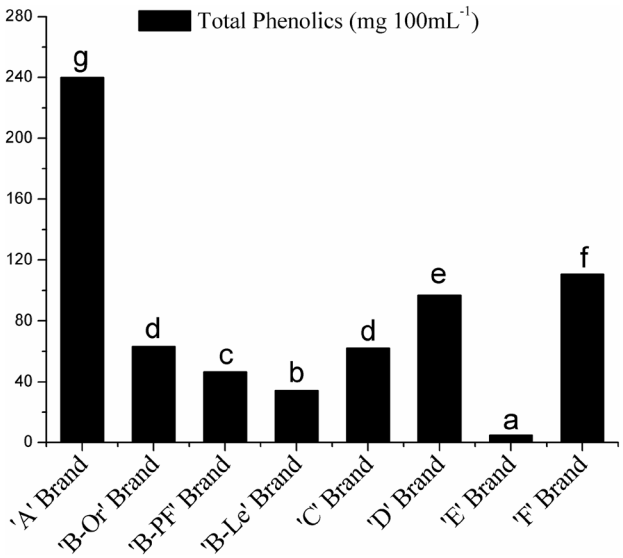

Fig. 7 Total phenolics mean values (mg 100 mL^{-1}) of six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott-Knott test at 5% significance level

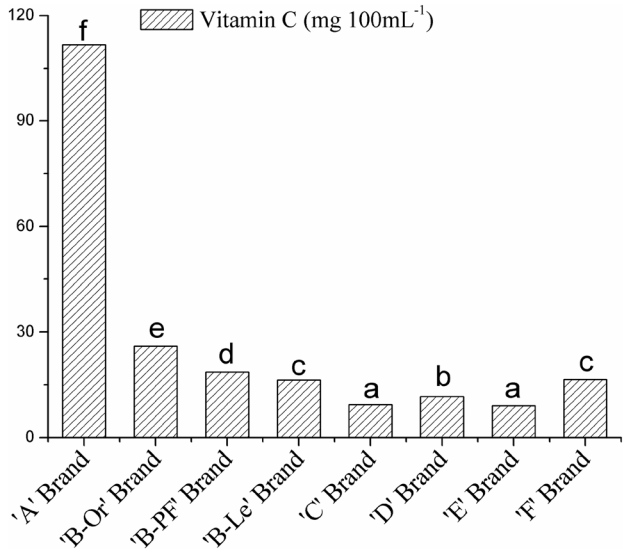

Fig. 8 Vitamin C (mg 100 mL^{-1}) mean values of six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott-Knott test at 5% significance level

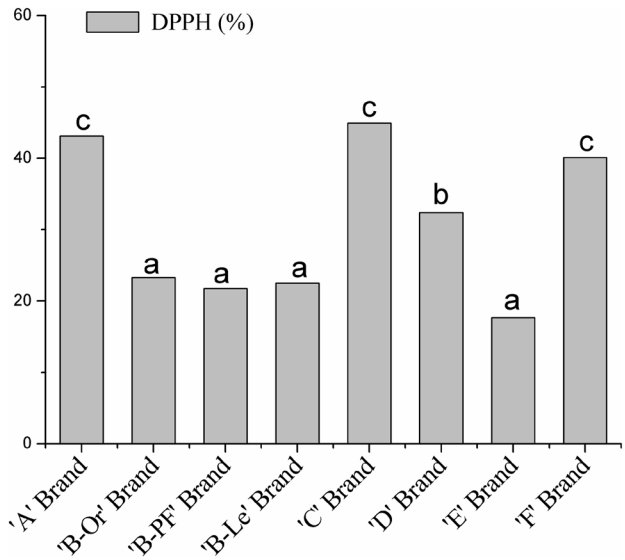

Fig. 9 DPPH (%) mean values of six brands of industrialized juices claiming 'natural.' Results were submitted to the Scott-Knott test at 5% significance level

brands that were equal to each other. The lowest averages were from the brands 'B-PF,' 'B-Le' and 'E' (Fig. 7).

Vitamin C content was also higher in the 'A' brand, followed by the 'B-Or,' 'B-PF,' 'B-Le,' 'F' and 'D' brands. The 'C' and 'E' brands had lower vitamin C averages (Fig. 8).

The antioxidant activity measured by the β-carotene/linoleic acid system was not statistically significant ($p > 0.05$), with mean values of 63.63% (data not shown). The sequestration of the $DPPH^{\cdot}$ radical was best performed by the 'A' (powder), 'C' (powder) and 'F' (ready-to-drink) drinks that were statistically the same. The 'D' brand obtained the second best result and the other brands were equal to each other (Fig. 9).

4 Discussion

From a microbiological point of view, the pH of beverages should not be higher than 4.5 because it favors the growth of *Clostridium botulinum* [16]. Except for 'E' brand, all other brands had a pH below 4.5, even those that sell their beverages in powdered form.

The high pH value and low acidity value of the 'E' brand could be justified by its ingredients and the beverage preparation mode that is taught by sellers. The information of ingredients is not available on their label which is a danger to consumers since the composition is not known. There are several vegetables that contain naturally anti-nutritional and/or toxic substances and depending on the amount ingested can represent serious health risks to those who consume them [17].

The total sugar content found in 'A,' 'B-Le' and 'D' brands was 22.84%, 42.20% and 16.54% higher than the label, respectively. The 'B-Or' brand 7 was 4% lower than the label. In the 'B-PF' and 'F' brands, the values found were the same as stated on their labels. Only the brand 'C' and 'E' do not declare the values of sugars. It is interesting to note that in almost all brands analyzed the soluble solids content was higher than the total sugar content. This can be attributed to the fact that soluble solids consist mostly of sugars, but other substances are soluble such as organic acids and mineral [18], which may have contributed to the increase of this variable.

The brand 'A' claims on its label that the drink is 'zero sugar'; however, according to National Health Surveillance Agency in Brazil (ANVISA-Agência Nacional de Vigilância Sanitária) Resolution (RDC) No. 360 [19], the non-significant amount of carbohydrate present in a serving of food must be less than or equal to 0.5 g in order to use the term 'does not contain' or 'zero' and, the sugar value found in this brand was higher than that established by ANVISA. The 'C 'brand, in turn, has lower sugar values than established by RDC No. 360. This brand declares that its solid compound is 'low calorie,' since it has 3.5 kcal, using correctly this information, since the aforementioned resolution determines that portions of foods with caloric values less than or equal to 4 kcal can use the statement 'zero' or 'does not contain' in your label.

The 'E' brand is a preparation of dehydrated vegetables, according to sellers, which gives the drink a green color. The brand 'C' has in its constitution *Camelia sinensis* in the form of green tea powder responsible for its yellowish green coloration.

Chlorophyll is the main pigment in green vegetables and some fruits [20]. It has been linked to prominent human health benefits; however, many other studies have been hampered by the lack of an overall structural analysis of all chlorophyll derivatives emerging in biological metabolism, tissue senescence or during food processing [21]. Carotenoids confer yellow coloration in fruits, vegetables and oils, and they are bioactive compounds that have the function of provitamin A (β-carotene and β-cryptoxanthin), antioxidant activity and prevent age-related macular degeneration and cataract (lutein) formation [22]. They are highly appreciated pigments as functional components, both for their coloring properties and human health benefits. However, some pigments belonging to the chlorophyll and carotenoid groups are thermolabile and, traditional thermal treatments of sterilization or high pasteurization of solid food products can induce their degradation [23].

The high content of phenolic compounds in 'A' brand is attributed to its ingredients (dehydrated cabbage powder, dehydrated spinach powder, orange pulp, blueberry pulp, apple pulp and dehydrated parsley—Table 1), the manner in which they were used; dehydrated and pulp that provide higher concentration of plant constituents and other non-plant ingredients such as vitamins added to the formulation, as these can also be quantified by the Folin–Ciocalteu test. The cabbage [24, 25], spinach [26], orange [27], *blueberry* [28], apple [29] and parsley [30] have been reported to be rich in phenolic compounds and antioxidants.

The antioxidant action, generally in these compounds, is due to the oxide-reduction potential of certain molecules, the ability to compete for active sites and receptors in the various cell structures, and also in the modulation of expression of genes encoding proteins involved in intracellular defense mechanisms against degenerative oxidative processes of cellular structures [31]. Studies have shown that consumption of fruits and vegetables rich in antioxidant compounds and polyphenols has been shown to prevent oxidation of cholesterol and other lipids in the arteries, increase endothelial prostacyclin formation, which inhibits platelet aggregation and reduces vascular tone, contributing to reduction in blood pressure and prevention of the development of cardiovascular diseases [32].

Only the brand 'A' declares the amount of vitamin C on its label (45 mg 100^{-1}), but the value found was higher than reported. This difference can be attributed to the fact that ascorbic acid was added to this drink and, even with the processing of the vegetables used, there may have been a residual amount that contributed to increase the value of vitamin C. Only the brand 'A' meets, if consumed only half of what is indicated on the label, the recommendation of daily intake recommended by the Institute of Medicine [33], where 75 mg is indicated for women, 90 mg for men and 45 mg for children. Following the preparation

recommendation indicated on the packaging of this same brand (20 g 200 mL^{-1} juice), a woman, a man and a child would consume 1.98, 1.5 and 3.97, respectively, times more vitamin C than recommended. Importantly, the 'A' brand claims on its packaging that the product is detox, which may induce consumers to adopt it as a convenient way to drink a healthy drink, however, due to the excess of vitamin C in its composition, its formulation should be reviewed and consumers, especially those with a predisposition to kidney problems, should avoid ingesting this drink.

Folchetti et al. [34] reported that vitamin C intake decreased the concentration of oxLDL inflammatory markers. Plasma concentrations of this particle are desirable for prevention of cardiovascular disease such as atherosclerosis. However, excessive vitamin C intake has been associated with oxalose, a disease characterized by calcium oxalate deposition in tissues. Calcium oxalate is a highly insoluble salt, corresponding to an end product of carbohydrate metabolism, vitamin C and some amino acids [35]. Oxalose is divided into two types and secondary may occur due to increased oxalate intake (ascorbic acid/vitamin C, ethylene glycol, methoxyflurane and oxalate-rich foods such as rhubarb, spinach, beet, chocolate and black tea), increased oxalate metabolism and reduced oxalate excretion. Vitamin C or ascorbic acid is readily converted to oxalate and it is believed that this may result in a hyperoxaluric state. Although there are controversies about its relationship with stone formation, cases of oxalate nephropathy have been reported with vitamin C intake < 2 g/day, although 2 g/day was considered a safe upper limit [34, 36, 37]. Among the ingredients of the 'A' brand, spinach is an oxalate-rich food [37].

The brands 'A' and 'F' obtained the highest values of total phenolics and vitamin C, and these compounds have antioxidant activity [38]. The *Camelia sinensis*, present in the 'C' and 'F' brands, is reported to be rich in catechins, phenolic compounds, which can represent more than 30% of the dry weight of the leaf [39].

Martins et al. [40] showed in their review on the in vivo antioxidant activity of phenolic compounds that this subject is one of the 'hot topics' among the scientific community; however, in vitro studies are the most common. However, according to the same authors, these studies do not consider the necessary biochemical, metabolic and physiological parameters, since both synthetic and natural antioxidants undergo numerous biochemical reactions during ingestion, digestion and absorption by the body. Therefore, and despite current advances, the effective bioavailability of different antioxidants is not clearly defined: While many of them are ingested in their active form, others need to be metabolized to become biologically active or even inactive, in addition, the co-ingestion of other nutrients as well as many endogenous factors and inter- and intra-individual variations affect their availability relative to the ingested dose. These facts explain why some plant species and even their isolated compounds do not show positive effects through in vitro studies, but a strong antioxidant potential is observed when in vivo studies are performed and vice versa [40].

The total dietary fiber content found in the 'D' and 'F' brands was 0.1 and 2.47%, respectively, values close to those described on their labels, considering a portion of 100 mL. Other brands declare no significant amounts of dietary fiber. Adequate dietary fiber intake seems to reduce the risk of developing some chronic diseases such as coronary artery disease, stroke, high blood pressure, diabetes mellitus and some gastrointestinal disorders. In addition, increased fiber intake improves serum lipid levels, lowers blood pressure levels, improves glycemic control in patients with diabetes mellitus, assists in reducing body weight and even improves immune system [41]. Current recommendations for dietary fiber intake vary according to age, sex and energy intake, with an appropriate recommendation being around 14 g of fiber for every 1000 kcal ingested [33, 41]. Therefore, both drinks can contribute to the daily diet of dietary fiber.

5 Conclusion

The beverages analyzed are in accordance with the literature for acidity, except the 'E' brand. The sugar content found in three brands ('A,' 'B-Le' and 'D') was higher than that stated on the labels, and the 'A' brand misleads the consumer by reporting that their drink is 'zero sugar.' In general, the drinks showed yellowish color, except the 'E' brand with greenish coloration. The brand 'A' also had higher levels of vitamin C than established by law. The amount of phenolic compounds was higher in the 'A' and 'F' brands. Antioxidant content was higher in 'A,' 'C' and 'F' brands. The 'D' and 'F' brands were shown as sources of dietary fiber. The content of some brands analyzed is in disagreement with their labels, thus inducing the consumer to error at the time of purchase, which represents a danger to consumers with some kind of food restriction.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest.

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